

Hypolipideimic and Hypocholestermic Effect of Pine Nuts in Rats Fed High Fat, Cholesterol-Diet

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Abstract: Pine nuts are cholesterol-free and good source of nutrients. It is rich in energy and consists of protein, carbohydrates, fatty acids, vitamins and minerals. The present study was carried out to investigate the hypolipideimic and hypocholestermic effect of pine nuts on rats fed high fat, cholesterol-diet. Rats were divided into five groups of seven rats each. Group (1) kept as negative control group; the remaining four groups fed high fat, cholesterol diet. Group (2) kept as positive control group; groups (3), (4) and (5) fed diets supplemented with 5, 10 and 15% pine nuts, respectively. Data illustrated that positive control group had significant reduction in food intake and no significant change in body weight gain. It also had significant increase in serum levels of total lipid (TL), triglycerides (TG), total cholesterol (TC), LDL-C, VLDL-C, AST, ALT and ALP and had significant decrease in serum level of HDL-C and value of HDL-C/TC ratio, compared to the negative control group. In contrast, supplemented diet with pine nuts caused significant reduction in food intake and non significant change in body weight gain. In addition to, a significant decrease in serum levels of the above mentioned parameters as well as significant increase in serum level of HDL-C and values of HDL-C/TC ratio, except low level of pine nuts (5%) induced no significant change in TG and HDL-C, as compared the positive control group. Normal histological structure was observed in heart of treated rats with 10 and 15% pine nuts and in aorta of treated rats with 15% pine nuts. It is concluded that pine nuts increased the reduction in lipid profile, lipoprotein cholesterol and liver enzymes. These decreases were increased with increase pine nut level.

Key words: Rats • Hypercholesterolemia • Hypertriglyceridemia • Serum lipoproteins • Pine nuts

INTRODUCTION

Pinus pinea trees (*Pinaceae* family) are grown widely in Europe and North America. Pine nuts are the edible seeds of pines. About 20 species of pine produce seeds large enough to be worth harvesting; in other pines the seeds are also edible, but are too small to be of great value as a human food [1]. Pine nuts are eaten raw or roasted; they are included as ingredients in a variety of traditional dishes such as meat, fish and vegetable dish as well as chocolates and desserts [2]. Different pine nuts obtained from various species differ in size, nutritional / medicinal value and taste. However, consumers are usually not sophisticated enough to distinguish between nuts of different species, therefore the nuts are usually

lumped together in the commerce and referred to as pine nuts [3].

Pine nuts are cholesterol-free [3] and are a good source of nutrients. It is rich in energy (628kcal) and consists of protein (11.6 g/100g), carbohydrates (19.3 g/100g) and fatty acids (61 g/100g) [4]. The most abundant amino acids in pine nuts are tryptophan and histidine as compared to eggs [5]. Moreover, fatty acids composition of pine nuts are 85% unsaturated fatty acids and 15% saturated fatty acids. The most common unsaturated fatty acids are linolic (48.4%), oleic (25.50%) and pinelolic (14.90%) acids [6]. Pine nuts oil possess a wide therapeutic effect, such as antibacterial, antifungal, antiviral, antiseptic, antineuralgic, cholagogue, choloretic, diuretic, expectorant, hypertensive [7, 8]. Pine nuts also,

contain vitamins, particularly B1 and B2 and minerals, especially potassium and phosphorus. Apart from nutritional value, consumption of nuts aids health. The composition of pine nuts shows variation among species and even some subspecies depending on geographical and climatic conditions [9, 10].

Cardiovascular disease is the most common cause of death in industrialized countries, with high plasma triglyceride (TG) levels being suggested to be independent risk factor [11]. It is known, that plasma levels of TG are influenced by dietary composition, smoking, body weight and genetic factors. Similarly to the other risk factors, it is estimated that the contribution of genetic and environmental factors on plasma levels of TG is roughly the same [12]. Hypercholesterolemia is a problem disease for many societies and is a one of the major risk factors for the development of cardiovascular diseases, such as atherosclerosis and its complications, acute infarctation of the myocardium or hypertension [13-15]. In addition, there is a correlation between these diseases and lipid abnormalities, especially high level of plasma cholesterol and blood pressure [16].

Although the focus of research so far has been mainly on the vascular effects of hyperlipidemia, i.e. arteriosclerosis, it is now quite evident that hyperlipidemia exerts direct effects on the myocardium in addition to the development of atherosclerosis [17, 18]. The known lipid lowering drugs (fibrates, statins, bile acid sequestrants, etc.) regulate the lipid metabolism by different mechanisms, but also have many side effects in patients [19]. Therefore, the present study aimed to investigate the hypolipidemic and hypocholestermic effect of pine nuts on rats fed high fat, cholesterol-diet. This was achieved by measuring the serum concentration of total lipid, triglycerides, total cholesterol and its lipoprotein fractions, AST, ALT and ALP, as well as histopathological examination of heart, aorta and liver.

MATERIALS AND METHODS

Rats and Diet: Thirty five male Sprague-Dawley rats weighing 200 ± 5 g were purchased from the Laboratory Animal Colony, Ministry of Health and Population, Helwan, Egypt. Cholesterol and basal diet constituents were obtained from El- Gomhorya Company for Chemical and Pharmaceutical, Cairo, Egypt.

Pine Nuts: Pine nuts were obtained from local market, Cairo Egypt.

Chemicals: Kits for biochemical analysis of serum total lipids, triglycerides, total cholesterol, HDL-C, AST, ALT and ALP were purchased from the Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt.

Preparation of Basal Diet: The basal diet (AIN-93M) was prepared according to Reeves *et al.* [20]. Diet was formulated to meet the recommended nutrients levels for rats.

Experimental Design: The experiment was conducted on thirty five male rats weighing approximately 200 ± 5 g for 6 weeks. The animals were housed in healthy condition at room temperature ($21-23^{\circ}\text{C}$), with 40-60% humidity, exposed to a 12:12-h light-dark cycle and fed on the basal diet and water was provided *ad libitum* for one week before starting the experimental for acclimatization. After acclimatization period rats were divided into five groups of seven rats each: group (1) fed the basal diet and kept as negative control group (normal rats); the remaining four groups fed high fat, cholesterol containing - diets. Group (2) kept as positive control group; groups (3), (4) and (5) fed supplemented diets with 5, 10 and 15% pine nuts, respectively. Hyperlipidemia and hypercholestermia in rats was done according to the method of Balkan *et al.* [21]. In briefly, basal diet was formulated with 1% cholesterol, 2% sheep fat and 0.5% cholic acid to enhance the enteral absorption of lipids.

At the end of the experimental period (6 weeks), diets were withheld from experimental rats for 12-h and then rats were sacrificed. Blood samples were collected from the portal vein into dry clean centrifuge tubes. For serum separation, blood samples were left at room temperature to get clot and then centrifuged for 15 minutes at 3000 rpm. Serum was carefully aspirated using a needle and transfers into dry clean test tubes and kept frozen at -10°C until chemical analysis.

Organs such as liver, kidney and heart were removed and washed with saline solution, dried and then weighted to calculate relative organs weight to body weight. Heart, aorta and liver of all rats were kept in formalin solution (10%) for histopathological examination.

Food Intake and Body Weight Gain Assay: Food intake (FI) was calculated every other day. The biological value of the different diets was assessed by the determination of its effect on body weight gain (BWG) at the end of the experimental period using the following formulas:

BWG = Final Body Weight - Initial Body Weight

Lipid Profile and Lipoprotein Cholesterol Assay: Total lipid (TL) concentrations were determined colorimetric using spectrophotometer apparatus adjust at 520 nm as described by kit instructions (Randox Co. Ireland). Triglycerides (TG), total cholesterol (TC) and high density lipoprotein cholesterol (HDL-C) concentrations were determined using enzymatic methods as described in the instructions provided with the kits (Analyticon® Biotechnologies AG, Germany). The absorbance of the testes samples were read using spectrophotometer adjusted at 546 nm for TG and TC and 500 nm for HDL-C. Then ratio of high density lipoprotein cholesterol to total cholesterol ratio was measured.

Low density lipoprotein cholesterol (LDL-C) concentration was calculated by using formula of Friedwald *et al.* [22] and very low density lipoprotein cholesterol (VLDL-C) was calculated using the following equation:

$$\text{LDL-Cholesterol} = \text{Total cholesterol} - (\text{HDL-C} + \text{TG}/5) \\ \text{VLDL-c (mg/ dL)} = \text{TG}/5$$

Liver Functions Assay: Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities were determined using colorimetric methods as described in the kits instruction (Diamond Co, Hannover, Germany). The absorption of the test samples were read at 505nm for GOT and GPT and at 510nm for ALP.

Histopathological Examination: Heart, aorta and liver of the scarified rats were taken and immersed in 10% formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. Specimens were then cleared in xylol, embedded in

paraffin, sectioned at 4-6 microns thickness and stained with Heamtoxylin and Eosin stain for examination of the liver as described by Carleton [23].

Statistical Analysis: Data were expressed as mean ± standard error. In order to compare the groups, analysis of variance (ANOVA) was used. P < 0.05 values were considered to be statistically significant.

RESULTS

Food Intake and Body Weight: Effects of high fat, cholesterol-diet either only or supplemented with different levels of pine nuts on food intake and body weight gain in rats are shown in table 1. Results revealed that high fat, cholesterol-diet significantly reduced food intake (18.43±0.48 g/d) at p<0.05 as compared to free cholesterol-diet (23.43 ± 0.72 g/d). In addition, high fat, cholesterol-diets supplemented with 5, 10 and 15% pine nuts significantly (p<0.05) reduced food intake (16.14 ± 0.34, 14.71 ± 0.42 and 13.14 ± 0.40 g/d, respectively) as compared to high fat, cholesterol-diets (18.43 ± 0.48 g/d). Mean values of initial, final body weight for all animals were partially similar for all groups and not significantly change. Body weight gain of positive control group and treated groups with pine nut were no significant deference as compared to the normal control rats.

Relative Organs Weight to Body Weight: Data in table 2 reported that positive control rats fed high fat, cholesterol-diet had significant increase in relative liver, heart and kidneys to body weight at p<0.05 as compared to negative control rats fed basal diet. Groups fed high fat, cholesterol-diets supplemented with different levels of pine nuts had no significant changes in relative liver weight to body weight as compared to the positive

Table 1: Food intake, body weight in rats.

Groups	Parameters as Mean ± SE			
	Food intake (g/d)	Initial weight (g)	Final weight (g)	Body weight gain (g)
Negative group	23.43±0.72 ^a	205.00± 3.80 ^a	238.43±2.21 ^a	33.43 ±0.97 ^a
Positive group	18.43±0.48 ^b	205.00± 3.16 ^a	238.14±3.22 ^a	33.14±0.80 ^a
5% Pine nut	16.14±0.34 ^c	205.57±1.94 ^a	239.71±1.98 ^a	34.14 ±1.07 ^a
10% Pine nut	14.71±0.42 ^d	205.71± 2.20 ^a	238.43±1.21 ^a	32.71 ±1.48 ^a
15% Pine nut	13.14±0.40 ^e	205.57± 2.11 ^a	237.57±2.07 ^a	32.00±0.72 ^a

Means in each column with different superscript letters differ significantly at p<0.05.

A uses harmonic mean sample size = 7 rats.

Table 2: Relative organs weight to body weight in rats.

Groups	Parameters as Mean ± SE		
	Liver (g)	Kidneys (g)	Heart (g)
Negative group	2.70 ± 0.11 ^b	0.58 ± 0.02 ^{ab}	0.28 ± 0.01 ^c
Positive group	4.26 ± 0.26 ^a	0.61 ± 0.02 ^a	0.37 ± 0.01 ^a
5% Pine nut	4.38 ± 0.22 ^a	0.58 ± 0.01 ^{ab}	0.35 ± 0.01 ^{ab}
10% Pine nut	4.32 ± 0.15 ^a	0.56 ± 0.02 ^{ab}	0.33 ± 0.01 ^b
15% Pine nut	4.64 ± 0.12 ^a	0.54 ± 0.02 ^b	0.34 ± 0.01 ^{ab}

Means in each column with different superscript letters differ significantly at P <0.05.

A uses harmonic mean sample size = 7 rats.

Table 3: Concentrations of serum total lipid, triglycerides and total cholesterol in rats.

Groups	Parameters as Mean ± SE		
	Total lipid (mg/dL)	Triglycerides (mg/dL)	Total cholesterol (mg/dL)
Negative group	371.57 ± 1.17 ^d	114.29 ± 2.24 ^c	52.00 ± .53 ^d
Positive group	413.71 ± 1.85 ^a	130.00 ± 2.20 ^a	92.57 ± 3.01 ^a
5% Pine nut	393.86 ± 1.99 ^b	124.57 ± 1.81 ^{ab}	78.14 ± .80 ^b
10% Pine nut	381.86 ± 1.68 ^c	119.57 ± 1.46 ^{bc}	69.43 ± 1.62 ^c
15% Pine nut	374.00 ± 1.60 ^d	114.71 ± 1.54 ^c	55.57 ± 2.14 ^d

Means in each column with different superscript letters differ significantly at P <0.05.

A uses harmonic mean sample size = 7 rats.

Table 4: Concentrations of serum LDL-C, HDL-C and VLDL-C as well as HDL-C/TC ratio in rats.

Groups	Parameters as Mean ± SE			
	LDL-C (mg/dL)	HDL-C (mg/dL)	VLDL-C (mg/dL)	HDL-C/TC ratio
Negative group	8.57 ± 0.784 ^d	20.57 ± 0.10 ^a	22.94 ± 0.50 ^c	0.40 ± 0.02 ^a
Positive group	52.71 ± 3.68 ^a	13.86 ± 0.59 ^b	26.00 ± 0.44 ^a	0.15 ± 0.09 ^d
5% Pine nut	38.37 ± 0.89 ^b	15.14 ± 0.34 ^b	24.63 ± 0.51 ^b	0.19 ± 0.08 ^c
10% Pine nut	25.09 ± 1.92 ^c	20.71 ± 0.57 ^a	23.63 ± 0.35 ^{bc}	0.30 ± 0.02 ^b
15% Pine nut	11.49 ± 2.18 ^d	21.14 ± 0.74 ^a	22.94 ± 0.31 ^c	0.38 ± 0.02 ^a

Means in each column with different superscript letters differ significantly at P <0.05.

A uses harmonic mean sample size = 7 rats.

Table 5: Concentrations of serum of AST, ALT and ALP in rats.

Groups	Parameters as Mean ± SE		
	AST (U/L)	ALT (U/L)	ALP (U/L)
Negative group	14.27 ± 0.44 ^d	10.93 ± 0.48 ^d	33.30 ± 0.35 ^d
Positive group	25.21 ± 0.50 ^a	21.90 ± 0.63 ^a	48.42 ± 0.67 ^a
5% Pine nut	21.60 ± 0.38 ^b	15.76 ± 0.32 ^b	36.47 ± 0.50 ^b
10% Pine nut	17.19 ± 0.24 ^c	13.91 ± 0.38 ^c	35.64 ± 0.26 ^{bc}
15% Pine nut	14.97 ± 0.26 ^d	11.23 ± 0.30 ^d	34.52 ± 0.26 ^{cd}

Means in each column with different superscript letters differ significantly at P <0.05.

A uses harmonic mean sample size = 7 rats

control group. Relative kidneys weight to body weight of groups fed high fat, cholesterol-diet supplemented with 5 and 10% pine nuts were not significant change (0.58 ± 0.01 and 0.56 ± 0.02 g, respectively) as compared to

the positive control group (0.61 ± 0.02g). Whereas, high levels of pine nuts (15%) significantly (p<0.05) reduced relative kidneys weight to body weight (0.54 ± 0.02g) as compared to the positive control group (0.61 ± 0.02g).

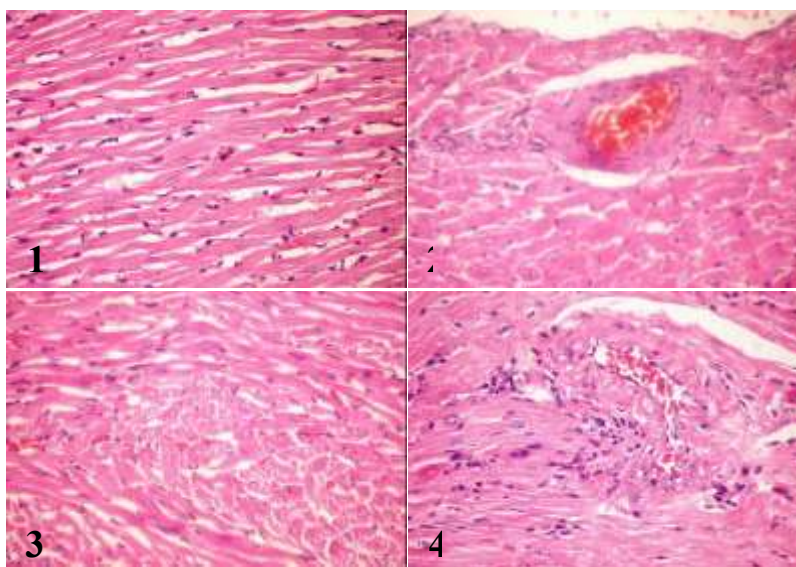
Administration of pine nuts at 5 and 15% caused no significant decrease in relative heart weight to body weight, while its level at 10 % caused significant decrease as compared to the positive control group.

Serum Concentrations of Total Lipid, Triglycerides and Total Cholesterol: Table 3 shows a significant increase ($p < 0.05$) in serum concentrations of total lipid (TL), triglycerides (TG) and total cholesterol (TC) in positive control group (413.71 ± 1.85 , 130.00 ± 2.20 and 92.57 ± 3.01 mg/dL, respectively) as compared to the negative control group (371.57 ± 1.17 , 114.29 ± 2.24 and 52.00 ± 0.53 mg/dL, respectively). Rats fed high fat, cholesterol-diets supplemented with 5, 10 and 15% pine nuts had lower mean \pm SE values for serum concentrations of TL, TG and TC, which were decreased significantly at $p < 0.05$, except treated group with 5% had no significant change in serum level of TG as compared the positive control group.

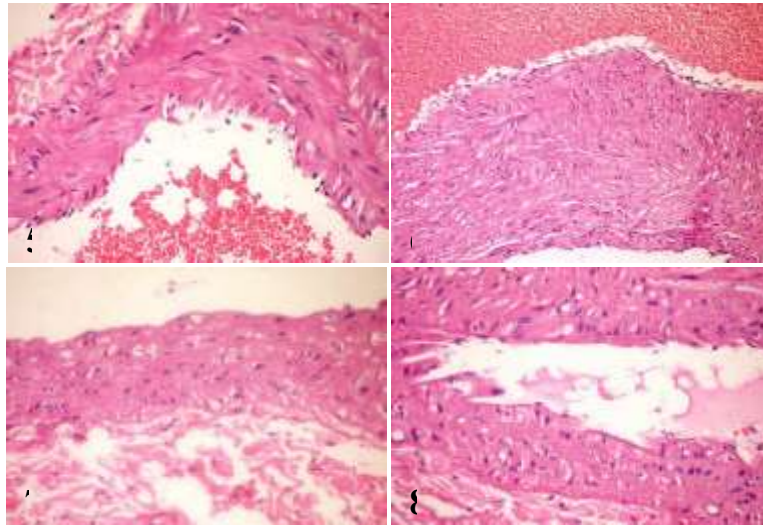
Serum Concentrations of LDL-C, HDL-C, VLDL-C as Well as HDL-C /TC Ratio: The effect of high fat, cholesterol-diet on serum concentrations of LDL-C, VLDL-C and HDL-C are shown in table 4. Results revealed that positive control group had significant increase in

serum level of LDL-C and VLDL-C mg/dL and significant decrease in serum levels of HDL-C, ($p < 0.05$) compared to negative control group. Mean \pm SE value of HDL-C/TC ratio significantly reduced (0.15 ± 0.09) in positive control group as compared to the normal control group (0.40 ± 0.02). Rats fed high fat, cholesterol-diets supplemented with different levels of pine nuts had significant reduce ($p < 0.05$) in serum levels of LDL-C and VLDL-C mg/dL as compare to positive rats. Low level of pine nuts (5%) produced no significant change in serum level of HDL-C, while 10 and 15% pine nuts caused significant increase, compared to positive control group. Mean \pm SE values for HDL-C/TC ratio were significantly increase ($p < 0.05$) in treated group with different levels of pine nuts as compared to positive group.

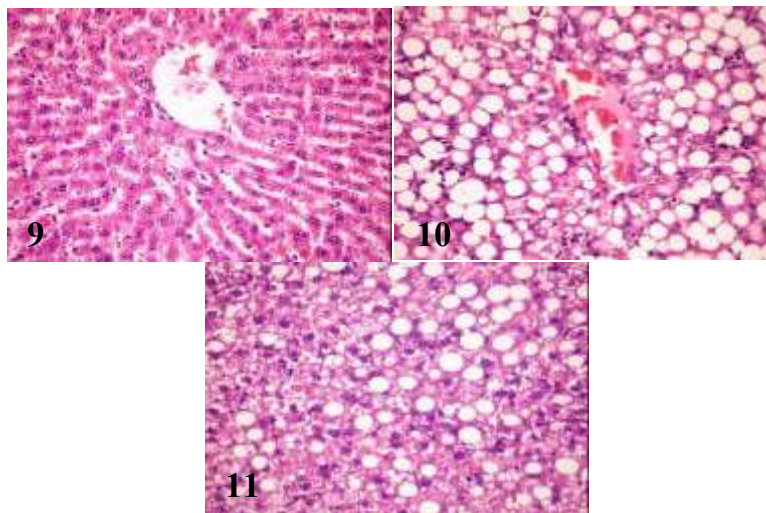
Serum Concentrations of AST, ALT and ALP: Table 5 shows significantly ($p < 0.05$) higher serum AST, ALT and ALP (25.21 ± 0.50 , 21.90 ± 0.63 and 48.42 ± 0.67 U/L, respectively) levels in positive control rats as compared to the negative control rats (14.27 ± 0.44 , 10.93 ± 0.48 and 33.30 ± 0.35 U/L, respectively). Administration of different levels of pine nuts resulted in a significant reduction in the level of serum AST, ALT and ALP ($p < 0.05$) as compared to positive control group.



- Fig. 1: Heart of rats from negative control group showing no histopathological changes (H and E x 400).
- Fig. 2: Heart of rats from positive control group showing congestion of cardiac blood vessel and hyalinosis of its wall (H and E x 400).
- Fig. 3: Heart of rats from positive control group showing granularity of the sarcoplasm of focal cardiac myocytes (H and E x 400).
- Fig. 4: Heart of rats from treated group with 5% pine nuts showing focal necrosis of myocytes associated with leukocytic cells infiltration and congestion myocardial blood vessels (H and E x 400).



- Fig. 5: Aorta of rats from negative control group showing no histopathological changes (H and E x 400).
Fig. 6: Aorta of rats from positive control group showing proliferation of smooth muscles of tunica media associated with thickening (H and E x 200).
Fig. 7: Aorta of rats from positive control group showing vacuulations of tunica media (H and E x 400).
Fig. 8: Aorta of rats from treated group with 5 and 10% pine nuts showing vacuulations of tunica media (H and E x 200).



- Fig. 9: Liver of negative control rats showing the normal histological structure of hepatic lobular (H and E x 400).
Fig. 10: Liver of positive control rats showing fatty change of hepatocytes (H and E x 400).
Fig. 11: Liver of treated rats with 5, 10 and 15% pine nuts showing fatty change of hepatocytes (H and E x 400).

Histopathological Examination

Heart: Heart sections of normal rats show normal histological structure as shown in figure 1. In contrast, heart sections of rats fed high fat, cholesterol-diet revealed congestion of cardiac blood vessel and hyalinosis of its wall (Figure2), in addition to granularity of the sarcoplasm of focal cardiac myocytes (Figure 3). Focal necrosis of myocytes associated with leucocytic cells infiltration and congestion myocardial blood vessels

were showed in examined heart sections of rats fed high fat, cholesterol-diet supplemented with 5% pine nut (Figure 4). Whereas, heart sections of rats fed high fat, cholesterol-diet supplemented with 10 and 15% pine nuts showed normal histological structure.

Aorta: Histopathological examination of aorta sections of negative control rats revealed no histopathological changes as shown in figure 5. However, proliferation of

smooth muscles of tunica media associated with thickening were observed in aorta of rats from positive control group fed high fat, cholesterol-diet (Figure 6), in addition to vacuulations of tunica media (Figure 7). Aorta sections of rats fed high fat, cholesterol-diet supplemented with 5 and 10% pine nuts had vacuulations of tunica media (Figure 8). However, rats fed diet supplemented with 15% pine nuts show normal histological structure.

Liver: Histopathological examination of livers from negative control rats revealed normal histological structure of hepatic lobule as shown in Figure 9. Liver sections of positive rats had cytoplasmic vacuolization of hepatocytes and fatty changes of hepatocytes (Figure 10). Fatty changes of hepatocytes were showed in examined liver sections of all groups fed diet supplemented with 5 and 10 and 15% pine nuts (Figure 11).

DISCUSSION

The present study was carried out to investigate the hypolipideimic and hypocholestermic effect of pine nuts at different levels on rats fed high fat, cholesterol diet.

The present results revealed that high fat, cholesterol-diet significantly reduce food intake, compared to normal basal diet. These results may be attributed to the higher caloric content of high fat, cholesterol diet as compared to normal basal diet. The increased in fat content of the diet are responsible for satiety and increased total calories. This result agreed with Sheyla *et al.* [24] who demonstrated that high fat content diet, which is used to induce hypercholesterolemia, leads to lower ingestion by the animals and induces malnutrition.

In relation to relative liver and heart weight to body weight, there was a significant increase in hypercholesterolemic group as compared to normal group. These results might be explained based on the accumulation of fat in the liver and heart cells. These results were confirmed by histopathological examination which showed that fatty changes of hepatocytes and granularity of the sarcoplasm of focal cardiac myocytes. These results were in accordance with Sheyla *et al.* [24] who reported that the increase in liver weight could be a consequence of their higher fat content (fat/liver). In addition to Puskas *et al.* [25] showed that intracellular lipid accumulation in cardiomyocytes in response to cholesterol diet.

The present results showed that positive control group had significant increase in the serum level of total lipid (TL), triglycerides (TG) and total cholesterol (TC), LDL-C, VLDL-C, however, a significant decrease in serum HDL-C, compared to the negative control group. The increases in serum concentrations of the above mentioned parameters and the reduction in serum HDL-C as results of high fat, cholesterol diet have been pointed out as risk factors for the development of atherosclerosis and related cardiovascular diseases, which was represented by the decrease in HDL-C/TC ratio. These results were confirmed by histopathological examination of heart which revealed congestion of cardiac blood vessel and hyalinosis of its wall and granularity of the sarcoplasm of focal cardiac myocytes. In addition to, aorta sections had proliferation of smooth muscles of tunica media associated with thickening and vacuulations of tunica media examined aorta. The present results agreed with Gregorio *et al.* [26] who showed that serum triglycerides, cholesterol, LDL-C, VLDL-C increased significantly, however, serum HDL-C decreased significantly in rats fed high cholesterol diet, compared to control rats. In addition to, Cohn [27] revealed that hyperlipidemia in rats is characterized by the rise in triglyceride-rich lipoproteins after a rich fat meal and it is associated with an increased risk of coronary artery disease. Triglyceride-rich lipoproteins can penetrate the artery wall and may have a direct effect on atherosclerosis. Zhen-Yu and Xiao-Qiand [28] demonstrated that lipid metabolism in rats feed high fat diet presented disorder and the level of serum TC and triglycerides increased significantly, compared with those in the normal control group.

Clinical studies indicated that hypercholesterolemia is an essential risk factor for coronary artery disease (CAD), where low-density lipoprotein (LDL) cholesterol plays a major role in the atherosclerosis and pathogenesis of CAD and other vascular diseases [29]. These results confirmed by Kumar *et al.* [30] who reported that rats fed high fat diet had significant elevation in plasma total and LDL-cholesterol and triglyceride and the ratio of HDL-C/total cholesterol was significant decrease in positive group, compared the normal control group.

The apparent results might be explained by the activity of lipoprotein lipase as reported by Tebib *et al.* [31] who found that activity of this enzyme augmented in hypercholesterolemic animals. Lipase transforms VLDL to LDL-cholesterol, what would lead to an increase in the serum concentration of LDL-C. On the other hand Berg *et al.* [32] demonstrated that uptake of LDL-cholesterol is dependent on receptors in plasmatic

membrane and these are in a reduced number when the cell has enough cholesterol. This may have happened in hepatic cells of the animal fed cholesterol-supplemented diets, explanatory their higher LDL-cholesterol concentration.

Assmann *et al.* [33] and Yarnell *et al.* [34] reported that hypertriglyceridemia increase the risk of acute coronary events and some clinical trials found high serum triglycerides to be an independent risk factor for cardiovascular disease. Furthermore, hypercholesterolemia combined with a marked hypertriglyceridemia leads to dysfunction in heart of rats [35].

Several studies showed that hyperlipidemia induces oxidative stress and this oxidative modification of lipoproteins in vessel walls play a key role in atherogenesis [36]. Hyperlipidemia exerts complex effects on the myocardium. Intracellular lipid accumulation in cardiomyocytes and several alterations in the structural and functional properties of the myocardium have been observed in response to cholesterol diet [25, 37].

Results also, recorded that positive control rats had significant elevation in serum level of AST, ALT and ALP, compared to the normal control rats. The elevations in the activity of liver enzymes may be attributed to their release from the cytoplasm into the blood circulation after rupture of the plasma membrane, cellular damage. These results confirmed by histopathological study, which observed that liver sections of had cytoplasmic vacuolization and fatty changes of hepatocytes. These results agreed with Tebib *et al.* [31] who showed that the activity of this enzyme increase in hypercholesterolemic animals. The uptake of LDL-cholesterol is dependent on receptors in plasmatic membrane. This may be happened in hepatic cells of the animal fed cholesterol-supplemented diets, explanatory their higher LDL-cholesterol concentration. Pincus and Schaffner [38] reported that this effect might be related to approximately 80% of AST in hepatocytes appears to be located in mitochondria, whereas ALT is thought to be predominantly no mitochondrial and it has been postulated that in mild hepatocellular injury. When the hepatocytes plasmatic but not the mitochondrial membrane is damaged, cytoplasmatic AST and ALT are released into serum with more severe hepatocellular injury and mitochondrial membrane damage.

With regard to the effect of pine nuts in rats fed high fat, cholesterol diet, results revealed that different levels of pine nuts reduce significantly food intake as compared to positive rats. It also reduced mean values of body weight gain and relative liver, heart and kidneys weight to body weight, compared to the positive control group.

The present results were noteworthy and might be due to the type of fatty acids composition in pine nuts especially pinolenic acid which is the main fatty acids in pine seed oil, or to some of its metabolites, because this unusual fatty acid is the main difference in the fat composition of the two experimental diets. These results agreed with Sugano *et al.* [39] and Matsuo *et al.* [40] who reported that pine nuts oil did not influence the growth performance of treated animals in comparison to other dietary fats. Asset *et al.* [6] found that body weight, food intake and liver weight were not significantly different between rats treated with pine nut oil and their respective controls. These results were confirmed with Yildiz-Gulay *et al.* [41] who reported that there was no change of body weight gain and average food intake in rabbits feed diet supplemented with pine nuts. The weights of the organs were similar and there were no significant differences in organ weights between control and treatment groups. Whereas, Ferramosca *et al.* [42] showed that pine nuts oil caused a significant reduction in body weight gain and liver weight. In addition to, Zhen-Yu and Xiao-Qiand, [28] revealed that the increase in body weight of treated rats with pine nuts oil was much less than that of the rats in high fat diet group. Furthermore, studies carried out in humans have demonstrated that pine nuts stimulates the release of the satiety gut hormones cholecystokinin (CCK) and glucagon like peptide-1 (GLP-1), thereby leading to a reduced feed intake. The suggestion that pine nuts could be used as an appetite suppressant in humans has therefore emerged [43].

The major finding of the present study was the reduction in serum levels of total lipid, triglycerides, total cholesterol, LDL-C, VLDL-C, AST, ALT and ALP, which were significant decrease and significant elevation in serum level HDL-C in treated rats, except low level of pine nuts (5%) induced non significant change in TG and HDL-C, compared to the positive control rats. It revealed that the lowest reduction of the above serum parameters were detected with increases the added level of pine nuts into high fat, cholesterol diet. Therefore, pine nuts may be decrease the incidence of atherosclerosis and coronary heart diseases, which was more detectable with the increase in serum level of HDL-C and HDL-C/TC ratio. These results were confirmed with the obtained results of histopathological examination.

The present results agreed with Sugano *et al.* [39] and Asset *et al.* [44] who showed that pine nuts supplementation lowers blood lipids and affects lipoprotein metabolism. Recent studies indicated that addition of pine nuts to the diet tended to decrease

plasma cholesterol concentrations [41] and caused no significant alterations on serum ALT, AST and ALP levels for rabbits [45].

The possible mechanism effect of pine nuts may be related to its fatty acid composition which is an important factor capable of modulating lipid metabolism. These results agreed with Asset *et al.* [6] who showed that levels of serum triglycerides were decreased significantly in the group supplemented with pine nuts oil compared to its respective control group. This reduction was accounted for by a decrease in VLDL-cholesterol.

In addition, it must be considered that other bioactive compounds present in the pine nuts diet may have a role in triggering the effects observed in this study. These results agreed with Wood *et al.* [46] who mentioned that pine nuts extract are rich in proanthocyanidins and contain a range of flavonoids including catechin, epicatechin, quercetin, dihydroquercetin, taxifolin and phenolic acids. Therefore, the improvement effects of pine nuts on lipid profile and lipoprotein cholesterol might be related to its antioxidant action. Previous research indicated that cardiovascular risk factors are associated with cognitive decline [47]. However, bioflavonoid consumption improved cardiovascular risk factors and promotes relaxation of vascular smooth muscle [48] and improvements of blood circulation to better cognitive functions [49]. Previous studies have shown that the potential effect of pine nuts for cardiovascular benefits includes improvement in endothelial function, reduction in plasma fibrinogen concentrations [50], reduced plasma viscosity and systolic blood pressure [51].

CONCLUSION

The present results revealed that the different levels of pine nuts caused reduction in food intake, body weight gain and reduced the incidence of hyperlipidemic and hypercholesteremic in rats fed high fat, cholesterol diet as well as liver enzymes concentrations. The lowest reduction was more detectable with increased the level of pine nuts.

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